

CLAIMS

We claim:

- 5 1. A method for detecting the presence of a target nucleic acid comprising:
 - a) cleaving an invasive cleavage structure, said invasive
 cleavage structure comprising an RNA target nucleic
 acid; and
 - b) detecting the cleavage of said invasive cleavage structure.
- 10 2. The method of Claim 1, wherein cleaving is carried out by a cleavage
 agent.
3. The method of Claim 1, wherein said target nucleic acid comprises a first
15 region and a second region, said second region downstream of and contiguous to said
 first region.
4. The method of Claim 3, wherein said invasive cleavage structure
 comprises said target nucleic acid, a first oligonucleotide, and a second oligonucleotide,
20 wherein at least a portion of said first oligonucleotide is completely complementary to
 said first region of said first target nucleic acid, and wherein said second oligonucleotide
 comprises a 3' portion and a 5' portion, wherein said 5' portion is completely
 complementary to said second region of said target nucleic acid.
- 25 5. The method of Claim 4, wherein at least said portion of said first
 oligonucleotide is annealed to said first region of said target nucleic acid and wherein at
 least said 5' portion of said second oligonucleotide is annealed to said second region of
 said target nucleic.
- 30 6. The method of Claim 1, wherein said cleaving generates a non-target
 cleavage product.

7. The method of Claim 6, wherein said detecting the cleavage of said invasive cleavage structure comprises detecting said non-target cleavage product.

5 8. The method of Claim 4, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

10 9. The method of Claim 4, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

15 10. The method of Claim 1, wherein said detecting the cleavage of said invasive cleavage structure comprises detection of fluorescence.

11. The method of Claim 1, wherein said detecting the cleavage of said invasive cleavage structure comprises detection of mass.

20 12. The method of Claim 1, wherein said detecting the cleavage of said invasive cleavage structure comprises detection of fluorescence energy transfer.

25 13. The method of Claim 1, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.

14. The method of Claim 4, wherein said first oligonucleotide is attached to a solid support.

30 15. The method of Claim 4, wherein said second oligonucleotide is attached to a solid support.

16. The method of Claim 2, wherein said cleavage agent comprises a structure-specific nuclease.

17. The method of Claim 16, wherein said structure-specific nuclease
5 comprises a thermostable structure-specific nuclease.

18. The method of Claim 2, wherein said cleavage agent comprises an enzyme, wherein said enzyme comprises a heterologous functional domain, wherein said heterologous functional domain provides altered functionality in a nucleic acid cleavage
10 assay.

19. The method of Claim 18, wherein said enzyme comprises a 5' nuclease.

20. The method of Claim 19, wherein said 5' nuclease comprises a
15 thermostable 5' nuclease.

21. The method of Claim 18, wherein said enzyme comprises a polymerase.

22. The method of Claim 21, wherein said polymerase is altered in sequence
20 relative to a naturally occurring sequence of a polymerase such that it exhibits reduced DNA synthetic activity from that of the naturally occurring polymerase.

23. The method of Claim 21, wherein said polymerase comprises a
25 thermostable polymerase.

24. The method of Claim 23, wherein said thermostable polymerase comprises a polymerase from a *Thermus* species.

25. The method of Claim 23, wherein said *Thermus* species is selected from
30 *Thermus aquaticus*, *Thermus flavus*, *Thermus thermophilus*, *Thermus filiformus*, and *Thermus scotoductus*.

26. The method of Claim 18, wherein said heterologous functional domain comprises an amino acid sequence that provides an improved nuclease activity in said nucleic acid cleavage assay.

27. The method of Claim 18, wherein said heterologous functional domain comprises an amino acid sequence that provides an improved substrate binding activity in said nucleic acid cleavage assay.

28. The method of Claim 18, wherein said heterologous functional domain comprises an amino acid sequence that provides improved substrate specificity in said nucleic acid cleavage assay.

29. The method of Claim 18, wherein said heterologous functional domain comprises two or more amino acids from a polymerase domain of a polymerase.

30. The method of Claim 29, wherein at least one of said two or more amino acids is from a palm region of said polymerase domain.

31. The method of Claim 29, wherein at least one of said two or more amino acids is from a thumb region of said polymerase domain.

32. The method of Claim 29, wherein said polymerase comprises *Thermus thermophilus* polymerase.

33. The method of Claim 29, wherein said two or more amino acids from said polymerase domain comprise two or more amino acids from amino acids 300-650 of SEQ ID NO:1.

34. The method of Claim 29, wherein said enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:2641-2674, 2710, 2712,

2714, 2717, 2719, 2722, 2724, 2726, 2728, 2730, 2732, 2734, 2736, 2738, 2740, 2742,
2744, 2746, 2748, 2750, 2752, 2754, 2756, 2758, 2760, 2762, 2766, 2768, 2770, 2772,
2774, 2776, 2778, 2780, 2781, 2782, 2783, 2785, 2787, 2789, 2791, 2793, 2795, 2797,
2799, 2801, 2803, 2805, 2807, 2809, 2811, 2813, 2815, 2817, 2819, 2821, 2823, 2825,
5 2827, 2829, 2831, 2833, 2835, and 2837.

35. The method of Claim 29, wherein said enzyme is encoded by a nucleic acid selected from the group consisting of SEQ ID NOs:2675-2709, 2711, 2713, 2715, 2716, 2718, 2720, 2721, 2723, 2725, 2727, 2729, 2731, 2733, 2735, 2737, 2739, 2741, 10 2743 2745, 2747, 2749, 2751, 2753, 2755, 2757, 2759, 2761, 2763, 2764, 2765, 2767, 2769, 2771, 2773, 2775, 2777, 2779, 2784, 2786, 2788, 2790, 2792, 2794, 2796, 2798, 2800, 2802, 2804, 2806, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2822, 2824, 2826, 2828, 2830, 2832, 2834, and 2836.

36. The method of Claim 8, further comprising the steps of forming a second invasive cleavage structure comprising said non-target cleavage product and cleaving 15 said second invasive cleavage structure.

37. The method of Claim 36, wherein said invasive cleavage structure or said second invasive cleavage structure comprises an oligonucleotide comprising a sequence 20 selected from the group consisting of SEQ ID NO:2837-4004.

38. The method of Claim 37, wherein said cleavage agent comprises enzyme having an amino acid sequence selected from the group consisting of SEQ ID NOs:2641- 25 2674, 2710, 2712, 2714, 2717, 2719, 2722, 2724, 2726, 2728, 2730, 2732, 2734, 2736, 2738, 2740, 2742, 2744, 2746, 2748, 2750, 2752, 2754, 2756, 2758, 2760, 2762, 2766, 2768, 2770, 2772, 2774, 2776, 2778, 2780, 2781, 2782, 2783, 2785, 2787, 2789, 2791, 2793, 2795, 2797, 2799, 2801, 2803, 2805, 2807, 2809, 2811, 2813, 2815, 2817, 2819, 2821, 2823, 2825, 2827, 2829, 2831, 2833, 2835, and 2837.

39. The method of Claim 1, wherein said RNA target nucleic acid comprises a cytochrome P450 RNA.

40. The method of Claim 1, wherein said RNA target nucleic acid comprises a cytokine RNA.

41. The method of Claim 3, wherein a portion of said target nucleic acid consisting of said first region and said second region of said target nucleic acid comprises a splice junction.

42. The method of Claim 3, wherein said first region or said second region of said target nucleic acid comprises an exon.

43. The method of Claim 3, wherein said first region or said second region of said target nucleic acid comprises an intron.

44. The method of Claim 1, wherein said RNA target nucleic acid is provided in a cell lysate.

45. The method of Claim 6, wherein said first oligonucleotide is covalently attached to said second oligonucleotide.

46. A kit for performing an invasive cleavage assay comprising a probe oligonucleotide and an invasive oligonucleotide configured to form an invasive cleavage structure in the presence of an RNA target nucleic acid.

47. The kit of Claim 46, further comprising a polypeptide encoded by a nucleic acid selected from the group consisting of SEQ ID NOs:2675-2709, 2711, 2713, 2715, 2716, 2718, 2720, 2721, 2723, 2725, 2727, 2729, 2731, 2733, 2735, 2737, 2739, 2741, 2743, 2745, 2747, 2749, 2751, 2753, 2755, 2757, 2759, 2761, 2763, 2764, 2765, 2767, 2769, 2771, 2773, 2775, 2777, 2779, 2784, 2786, 2788, 2790, 2792, 2794, 2796,

2798, 2800, 2802, 2804, 2806, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2822, 2824, 2826, 2828, 2830, 2832, 2834, and 2836.

48. The kit of Claim 47, wherein said polypeptide is selected from the group consisting of SEQ ID NOs:2641-2674, 2710, 2712, 2714, 2717, 2719, 2722, 2724, 2726, 2728, 2730, 2732, 2734, 2736, 2738, 2740, 2742, 2744, 2746, 2748, 2750, 2752, 2754, 2756, 2758, 2760, 2762, 2766, 2768, 2770, 2772, 2774, 2776, 2778, 2780, 2781, 2782, 2783, 2785, 2787, 2789, 2791, 2793, 2795, 2797, 2799, 2801, 2803, 2805, 2807, 2809, 2811, 2813, 2815, 2817, 2819, 2821, 2823, 2825, 2827, 2829, 2831, 2833, 2835, and 2837.

49. The kit of Claim 46, wherein said kit comprises an oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:2837-4004.

50. The kit of Claim 47, wherein said kit comprises an oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:169-211 and 619-706, 709-2640 and 2837-4004.

51. A method for detecting the presence of two or more target nucleic acid sequences comprising:

- a) cleaving two or more invasive cleavage structures, each of said two or more invasive cleavage structures comprising an RNA target nucleic acid having a target sequence, wherein each of said invasive cleavage structures comprises a different RNA target sequence; and
- b) detecting the cleavage of said invasive cleavage structure.

52. A composition comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:2675-2709, 2711, 2713, 2715, 2716, 2718, 2720, 2721, 2723, 2725, 2727, 2729, 2731, 2733, 2735, 2737, 2739, 2741, 2743, 2745, 2747, 2749, 2751, 2753, 2755, 2757, 2759, 2761, 2763, 2764, 2765, 2767, 2769, 2771, 2773, 2775, 2777, 2779, 2784, 2786, 2788, 2790, 2792, 2794, 2796, 2798, 2800, 2802, 2804, 2806,

2808, 2810, 2812, 2814, 2816, 2818, 2820, 2822, 2824, 2826, 2828, 2830, 2832, 2834, and 2836 and the complements thereof.

53. A vector comprising the nucleic acid sequence of Claim 52.

54. A host cell comprising the vector of Claim 53.

55. A composition comprising a polypeptide encoded by a nucleic acid selected from the group consisting of SEQ ID NOs:2675-2709, 2711, 2713, 2715, 2716, 2718, 2720, 2721, 2723, 2725, 2727, 2729, 2731, 2733, 2735, 2737, 2739, 2741, 2743, 2745, 2747, 2749, 2751, 2753, 2755, 2757, 2759, 2761, 2763, 2764, 2765, 2767, 2769, 2771, 2773, 2775, 2777, 2779, 2784, 2786, 2788, 2790, 2792, 2794, 2796, 2798, 2800, 2802, 2804, 2806, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2822, 2824, 2826, 2828, 2830, 2832, 2834, and 2836.

56. The composition of Claim 55, wherein said polypeptide is selected from the group consisting of SEQ ID NOs:2641-2674, 2710, 2712, 2714, 2717, 2719, 2722, 2724, 2726, 2728, 2730, 2732, 2734, 2736, 2738, 2740, 2742, 2744, 2746, 2748, 2750, 2752, 2754, 2756, 2758, 2760, 2762, 2766, 2768, 2770, 2772, 2774, 2776, 2778, 2780, 2781, 2782, 2783, 2785, 2787, 2789, 2791, 2793, 2795, 2797, 2799, 2801, 2803, 2805, 2807, 2809, 2811, 2813, 2815, 2817, 2819, 2821, 2823, 2825, 2827, 2829, 2831, 2833, 2835, and 2837.

57. A method for producing an altered enzyme with improved functionality in a nucleic acid cleavage assay comprising:

a) providing:

i) a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs:2641-2674, 2710, 2712, 2714, 2717, 2719, 2722, 2724, 2726, 2728, 2730, 2732, 2734, 2736, 2738, 2740, 2742, 2744, 2746, 2748, 2750, 2752, 2754, 2756, 2758, 2760, 2762, 2766, 2768, 2770, 2772, 2774, 2776, 2778, 2780, 2781, 2782, 2783, 2785, 2787, 2789, 2791, 2793, 2795, 2797, 2799, 2801, 2803, 2805, 2807,

2809, 2811, 2813, 2815, 2817, 2819, 2821, 2823, 2825, 2827, 2829, 2831, 2833, 2835,
and 2837;

ii) a nucleic acid encoding a polypeptide comprising a
sequence selected from the group consisting of SEQ ID NOs:2675-2709, 2711, 2713,
5 2715, 2716, 2718, 2720, 2721, 2723, 2725, 2727, 2729, 2731, 2733, 2735, 2737, 2739,
2741, 2743 2745, 2747, 2749, 2751, 2753, 2755, 2757, 2759, 2761, 2763, 2764, 2765,
2767, 2769, 2771, 2773, 2775, 2777, 2779, 2784, 2786, 2788, 2790, 2792, 2794, 2796,
2798, 2800, 2802, 2804, 2806, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2822, 2824,
2826, 2828, 2830, 2832, 2834, and 2836; and

10 iii) a nucleic acid test substrate;

b) introducing one or more heterologous domains into said nucleic
acid to produce an altered nucleic acid encoding an altered enzyme;

c) contacting said altered enzyme and said polypeptide with said
nucleic acid test substrate to produce cleavage products; and

15 d) comparing cleavage products produced by said altered enzyme to
cleavage products produced by said polypeptide.